

# Impact of Serum Homocysteine on Platelet Count in Stable Hemodialysis Patients

Hamid Nasri, MD

*Hemodialysis Section, Hajar Medical, Educational and Therapeutic Center, Shahrekord University of Medical Sciences, Shahrekord, Iran.*

**KEY WORDS:** platelet count, hemodialysis, end-stage renal failure, serum homocysteine

## ABSTRACT

**Objective:** The objective of this study was to elucidate whether and how the level of homocysteine as a cause of hemostatic abnormalities affects platelet count in hemodialysis patients with uremia.

**Methods:** A cross-sectional study was conducted on patients with end-stage renal disease (ESRD) who were undergoing maintenance hemodialysis treatment with acetate basis dialysate and polysulfone membranes. Serum total homocysteine (HCY), hemoglobin, platelet (PLT), and white blood cell (WBC) counts were measured. Levels of serum calcium (Ca), phosphorus (P), and magnesium (Mg) were also measured. There were 39 study patients (15 female, 24 male) with a mean age of  $46 \pm 18$  years.

**Results:** The duration of dialysis treatment was  $31 \pm 35$  months (median, 18 months). The value of serum homocysteine for all the patients was  $5 \pm 2.5$   $\mu\text{mol/L}$  (median, 4.5  $\mu\text{mol/L}$ ). The mean PLT count was  $155 \pm 77 \times 10^3$  u/L (median,  $158 \times 10^3$  u/L).

**Conclusion:** This study showed a significant positive correlation of PLT count with serum homocysteine ( $r=0.35$ ,  $P=0.044$ ). In hemodialysis patients high homocysteine levels make the platelets more likely to clump and cause clots and contribute to the possibility of thrombotic events among these patients.

## INTRODUCTION

Renal failure has classically been associated with a bleeding tendency. Thrombotic events are also common among patients with end-stage renal disease (ESRD). A variety of thrombosis-favoring hematologic alterations have been demonstrated in these patients. A non-traditional risk factor for thrombosis, such as hyperhomocysteinemia, is present in a significant proportion of chronic dialysis patients. Hemodialysis (HD) vascular access thrombosis and ischemic heart disease are well-recognized complications in these patients. Deep venous thrombosis and pulmonary embolism are viewed as rare in chronic dialysis patients, but recent studies suggest that this perception should be reconsidered.<sup>1</sup> Studies have shown that mild-to-moderate elevations in plasma total homocysteine (tHcy) levels are observed in the great majority (>85%) of patients with ESRD who are undergoing maintenance dialysis.<sup>2</sup>

Homocysteine (Hcy) is a non-pro-

tein, sulfur-containing amino acid that exists in various forms: a protein-bound fraction (70% to 80%), a free oxidized form (20% to 30%), and a free reduced form (approximately 1%) which recently has attracted considerable interest as it may, by several mechanisms, mediate premature atherosclerosis and cardiovascular disease (CVD). Indeed, in the general population, studies have shown that even mildly elevated plasma tHcy levels are associated with an increased cardiovascular risk.<sup>3-5</sup> The mechanism by which Hcy exerts these effects has yet to be fully elucidated, although a variety of possible mechanisms have been proposed, including endothelial dysfunction or hemostatic abnormalities. Its effects may be secondary to impaired fibrinolysis or increased platelet reactivity.<sup>6</sup> However, the influence of Hcy on platelets—cells central to the atherothrombotic process—has never been addressed directly in studies of hemodialysis patients.

This study was performed to elucidate whether and how the level of homocysteine as a cause of hemostatic abnormalities affects the platelet count in patients with uremia on maintenance hemodialysis.

## **PATIENTS AND METHODS**

### **Patients**

This cross-sectional study was conducted on patients with ESRD who were undergoing maintenance hemodialysis treatment with acetate basis dialysate and polysulfone membranes. The study was conducted in hemodialysis section of Hajar Medical, Educational and Therapeutic Center of Shahrekord University of Medical Sciences in Shahrekord, Iran, during July and August of 2005.

A total of 39 patients (15 female and 24 male) with a mean age of  $46 \pm 18$  years participated in the study. The study was carried out in hemodialysis

section of Hajar Medical, Educational and Therapeutic Center of Shahrekord University of Medical Sciences in Shahrekord of Iran. The duration of dialysis treatment was  $31 \pm 35$  months (median, 18 months). Exclusion criteria included active or chronic infection and use of non-steroidal anti-inflammatory drugs (NSAIDs) or angiotensin-converting enzyme (ACE) inhibitors or any other drug known to have adverse effects on platelet production or function.

According to the severity of secondary hyperparathyroidism, each patient being treated for secondary hyperparathyroidism was given oral active vitamin D3 calcitriol (Rocaltrol; Roche Laboratories Inc., Nutley, NJ), calcium carbonate capsule, and sevelamer (Renagel; Genzyme Europe BV, Naarden, The Netherlands) tablets at various doses. According to the severity of anemia, patients were prescribed intravenous (IV) iron therapy with Iron Sucrose (Venofer; International Inc. St. Gallen, Switzerland) at various doses after each dialysis session. All patients received 6 mg of folic acid daily, 500 mg of Acetyl- L-Carnitine (Jarrow Formulas, Inc., Los Angeles, CA) daily, oral vitamin B-complex tablets daily, and 2,000 U IV recombinant human erythropoietin (Eprex; Janssen-Cilag, CILAG- AG International Zug, Switzerland) after each dialysis session.

Table 1 summarizes the patients' mean age, length of time on hemodialysis, dialysis dosage, and the results of the laboratory tests.

### **Laboratory Methods**

Blood samples were collected after an overnight fast. All samples were centrifuged within 15 minutes of venipuncture and serum HCY was measured by enzyme-linked immunosorbent assay (ELISA) method using DRG kits (DRG Diagnostics, Berlin, Germany). The normal range for serum total homocysteine

**Table 1.** Mean  $\pm$  standard deviation (SD) and median values of age, duration, and doses of hemodialysis as well as laboratory results for all patients.

Total patients (n=39)	Mean $\pm$ SD	Median
Age (years)	46 $\pm$ 18	42
Duration of hemodialysis (months)	31 $\pm$ 35	18
Dialysis dose (sessions)	280 $\pm$ 381	156
URR %	58.7 $\pm$ 8.7	58
Ca (mg/dL)	7.7 $\pm$ 1	8
Hgb (g/dL)	9 $\pm$ 2	9
HCT %	28 $\pm$ 6	29
Homocysteine ( $\mu$ mol/L)	5 $\pm$ 2.5	4.5
Mg (mg/dL)	2.5 $\pm$ 04	2.4
P (mg/dL)	6.4 $\pm$ 1.9	6.4
WBC count (cells/mm <sup>3</sup> )	5864 $\pm$ 1922	5500
PLT count ( $\times 10^3$ u/L)	155 $\pm$ 77	158

URR= urea reduction rate; Ca=serum calcium; Hgb=hemoglobin; HCT= hematocrit; Mg= magnesium; P= phosphorus;WCB=white blood cell; PLT=platelet

is 25 to 125  $\mu$ mol/L. In addition, complete blood count containing hemoglobin (Hgb), hematocrit (Hct), platelet (PLT), and white blood cell (WBC) count were measured using Sysmex-KX-21N cell counter (Sysmex Corporation, Mundelein, IL). Levels of serum calcium (Ca), phosphorus (P), magnesium (Mg) were measured using standard methods. Duration and doses of hemodialysis treatment were calculated from the patients' records. The duration of each hemodialysis session was 4 hours. For the efficacy of hemodialysis, the urea reduction rate (URR) was calculated from pre- and post-blood urea nitrogen (BUN) data. Body mass index (BMI) calculated using the standard formula (postdialyzed weight in kilograms/height in meters squared; kg/m<sup>2</sup>).

### Statistical Analysis

Results are expressed as the mean  $\pm$  standard deviation (SD) and median values. Statistical correlations were assessed using a partial correlation test.

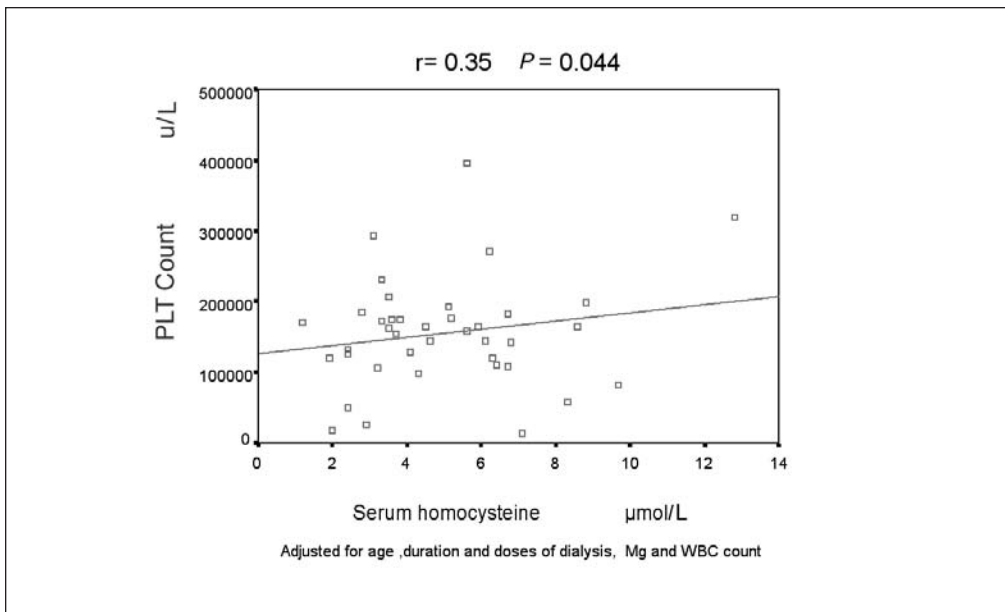
All statistical analyses were performed using SPSS version 11.00 (SPSS Inc. Chicago, IL). Statistical significance was defined as  $P < 0.05$ .

### RESULTS

The value of serum homocysteine for all patients was 5  $\pm$  2.5  $\mu$ mol/L (median, 4.5  $\mu$ mol/L). The mean PLT count was 155  $\pm$  77  $\times 10^3$ u/L (median, 158  $\times 10^3$ u/L). In this study a significant positive correlation of PLT count with serum homocysteine ( $r=0.35$ ,  $P=0.044$ ) adjusted for age, duration and doses of dialysis, WBC count, serum Mg was seen (Figure 1).

### DISCUSSION

In this study, a significant positive correlation of PLT count with serum homocysteine was found. Recent evidence indicates that hyperhomocysteinemia is an independent risk factor for atherosclerosis, thrombosis, and other cardiovascular diseases. This may be secondary to impaired fibrinolysis or increased platelet reactivity. Nitric oxide (NO), a



**Figure 1.** Significant positive correlation of platelet (PLT) count with serum homocysteine ( $r=0.35$ ,  $P=0.044$ ), adjusted for age, duration and doses of dialysis, white blood cell count (WBC), serum magnesium (Mg).

product from L-arginine by nitric oxide system (NOS) and potent anti-aggregating agent, plays an important role in the regulation of platelet function. The results of a study conducted by Leoncini et al indicate that the L-arginine/NO pathway is one of the various targets of homocysteine in human platelets. The increased  $\text{Ca}^{2+}$  levels associated with reduced NO formation may generate hyperactivation and may contribute to the thrombogenic processes.<sup>6</sup>

The pathogenic mechanism of atherosclerosis and thrombosis caused by homocysteine has not been fully clarified, but oxygen-free species produced by the homocysteine metabolism and auto-oxidation could play a role.<sup>7</sup> To study the effect of homocysteine on arachidonic acid release in human platelets, Signorello et al showed that homocysteine induces oxidative stress in human platelets in vitro.<sup>7</sup> The unbalance in platelet redox-state and the increased thromboxane  $\text{B}_2$  (TXB<sub>2</sub>) formation may generate hyperactivation, contributing to a thrombogenic state leading to cardio-

vascular diseases.<sup>7</sup>

It is thought that L-arginine/nitric oxide synthase/nitric oxide plays an important role in the regulation of platelet function.<sup>8</sup> Li et al showed that the L-arginine/NO pathway is involved in the mechanism responsible for the effects of HCY on platelets by diminishing NO production through decreased uptake of L-arginine and the resulting increased platelet reactivity.<sup>8</sup> In this regard, Mutus, in a study which included 28 patients with Type I (insulin-dependent) diabetes mellitus, 30 patients with Type II (non-insulin-dependent) diabetes mellitus, and 34 healthy subjects, showed that basal platelet NO production was lower in diabetic patients than in healthy subjects. NO release was reduced by in vitro homocysteine incubation, being lower in platelets from diabetic patients than in platelets from control subjects.<sup>9</sup> They concluded that homocysteine could exert its atherogenic action in healthy and diabetic subjects partly by inhibiting platelet nitric oxide production with the subsequent

increased platelet activation and aggregation.<sup>9</sup>

Using a rat model, Duranp et al showed that dietary folic acid deficiency, a major cause of basal hyperhomocysteinemia, is associated with enhanced macrophage-derived tissue factor and platelet activities.<sup>10</sup> They proposed that a homocysteine-induced oxidative stress may account for this hypercoagulable state. They concluded that moderate hyperhomocysteinemia plays a role in the development of a thrombogenic state that might be mediated by the occurrence of oxidative stress.<sup>10</sup>

Studies in the general population suggest that low-grade inflammation, endothelial dysfunction, and platelet activation are associated with an increased risk of cardiovascular events.<sup>10</sup> Landray et al attempted to assess the markers of inflammation, endothelial dysfunction, and platelet activation in 334 patients with chronic kidney disease compared to two age- and sex-matched control groups, one comprised of 92 patients with coronary artery disease and the other comprised of 96 apparently healthy individuals with no history of cardiovascular or kidney disease.<sup>11</sup> They found that chronic kidney disease is associated with low-grade inflammation, endothelial dysfunction, and platelet activation, even among patients with moderate renal impairment.<sup>11</sup>

In hemodialysis patients, folate deficiency and resultant hyperhomocysteinemia are commonly seen.<sup>12</sup> Our results show that, in hemodialysis patients, serum HCY had a positive correlation with PLT count that could explain some thrombotic events among patients with ESRD. The above-mentioned studies regarding the mechanisms for increased PLT activities through hyperhomocysteinemia may also exist in ESRD patients, therefore our conclusion indicates a need for greater attention to this issue in hemodialysis patients. They also

showed that more study is needed on the effects of serum HCY on PLT function in ESRD patients.

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